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IMPROVED TECHNIC OF AGGLUTINATION TEST IN TYPHOID FEVER—THE USE OF FORMALINIZED CULTURES.*

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AMONG the various methods employed in the serum test for typhoid fever two are especially popular and convenient of application. These are the dried-blood method, perfected and popularized by Wyatt Johnston, and the fresh-serum method, used by Widal. For laboratory and scientific purposes the fresh-serum method is preferable, as it offers a means of determining the maximum agglutinative power by making dilutions of various strengths. If the test is to be merely qualitative and for clinical purposes, and especially if the blood is sent by mail to a laboratory, the dried-blood method is by far the more convenient. Both methods are reliable and readily applied, but there is one drawback common to both. They both require a living culture of B. typhosus which should not be over twenty-four hours old, and this is not always obtainable except at institutions where the work is done on a large scale. Pröscher† has shown, however, that a twenty-four-hour bouillon culture of B. typhosus which has been killed with formalin gives the same specific reaction as the living culture when mixed with blood, or blood serum, from a typhoid-fever patient. It is this method which will be considered here.

Material for study was obtained from the cases of Dr. Billings and Dr. Herrick in the Presbyterian Hospital, and as typhoid fever was abundant during the summer and autumn of the year 1902, it afforded me an excellent opportunity to give the method a fair trial.

Technic.—About 500 c.c. of plain bouillon were inoculated with B. typhosus, incubated at 36° C. for twenty-four hours, and then the organisms were killed by adding 1 c.c. of formalin for every 100 c.c. of bouillon. A living twenty-four-hour bouillon culture was also kept on hand, and comparative

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† *Centralbl. f. Bakteriol.*, 1902, 31, p. 400.

tests were always carried out. The tests were made with fresh blood serum and with dried blood as follows: Five to 10 c.c. of blood were drawn from a superficial vein of the arm, allowed to clot in a sterile bottle, and placed in the refrigerator. The clear serum which exuded was taken from the clot after twelve to twenty-four hours and diluted with nine parts of distilled water, and the resulting mixture called a 1:10 dilution, each c.c. containing 0.1 c.c. of serum. Having made the 1:10 dilution of serum, small quantities were mixed with the living and also with the dead typhoid culture in the following manner:

1.0 c.c. 1:10 serum to 4.0 c.c. bouillon culture = 1:50
1.0 " " " " 5.0 " " " = 1:60
1.0 " " " " 6.0 " " " = 1:70
1.0 " " " " 7.0 " " " = 1:80
1.0 " " " " 8.0 " " " = 1:90
1.0 " " " " 9.0 " " " = 1:100
0.5 " " " " 9.5 " " " = 1:200
0.25 " " " " 9.75 " " " = 1:400

Now 1 c.c. of the 1:10 solution was mixed with 9 c.c. of distilled water, the dilution called 1:100, and the tests continued as follows:

1 c.c. 1:100 serum to 4 c.c. bouillon culture = 1:500
1 " " " " 5 " " " = 1:600
1 " " " " 6 " " " = 1:700

These tests were continued until a dilution of 1:100,000 was reached to determine the least possible quantity of serum required to produce visible agglutination, and to compare the results obtained with the living and dead culture, also comparing the results obtained with blood serum and dried blood. A control tube, made by mixing 1 c.c. of a 1:10 dilution of normal blood serum with 4 c.c. of the typhoid culture, was always used.

In this manner thirty-four cases were studied, using fresh blood serum. At the same time tests were carried out by the dried-blood method, with both living and dead culture. Four drops of blood were spread on a glass slide, allowed to dry, and later dissolved in 2 c.c. of distilled water, making approximately a 1:10 dilution. One c.c. of this dilution was put into each of two test-tubes *a* and *b*. To Tube *a* were added 4 c.c. of the living culture, and to *b*, 4 c.c. of the dead culture. The tubes were now set aside together with those containing fresh serum, and the results compared (see Table of Results).

The lowest dilution at which the test was considered positive was 1:50. The second figures under each case indicate the highest dilutions at which visible agglutination took place inside of twelve hours.

Of the thirty-four cases studied thirty gave positive reaction, and confirmed the clinical diagnosis of typhoid fever which in the

TABLE OF RESULTS.

Case	Dead Bacilli	Living Bacilli	Remarks
1......	1 : 50 agg. in 10 min.	1: 50 agg. in 10 min.	Typhoid fever
	1 : 5,000 agg. in 5 hrs.	1: 5,000 agg. in 9 hrs.	
2......	1 : 50 agg. in 15 min.	1: 50 agg. in 15 min.	Typhoid fever
	1 : 2,000 agg. in 6 hrs.	1: 2,000 agg. in 10 hrs.	
3......	1 : 50 agg. instantly	1: 50 agg. in 5 min.	Typhoid fever
	1 : 8,000 agg. in 5 hrs.	1: 8,000 agg. in 8 hrs.	
4......	1 : 50 agg. in 30 min.	1: 50 agg. in 40 min.	Typhoid fever
	1 : 600 agg. in 7 hrs.	1: 600 agg. in 12 hrs.	
5......	1 : 50 no agg.	1: 50 no agg.	Doubtful
6......	1 : 50 agg. in 30 min.	1: 50 agg. in 40 min.	Typhoid fever
	1 : 1,000 agg. in 8 hrs.	1: 1,000 agg. in 12 hrs.	
7......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 2,000 agg. in 6 hrs.	1: 2,000 agg. in 10 hrs.	
8......	1 : 50 agg. in 30 min.	1: 50 agg. in 35 min.	Typhoid fever
	1 : 5,000 agg. in 5 hrs.	1: 5,000 agg. in 8 hrs.	
9......	1 : 50 agg. in 30 min.	1: 50 agg. in 40 min.	Typhoid fever
	1 : 6,000 agg. in 5 hrs.	1: 6,000 agg. in 7 hrs.	
10......	1 : 50 no agg.	1: 50 no agg.	Paratyphoid
11......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 1,000 agg. in 6 hrs.	1: 1,000 agg. in 8 hrs.	
12......	1 : 50 agg. in 5 min.	1: 50 agg. in 10 min.	Typhoid fever
	1 : 2,000 agg. in 6 hrs.	1: 2,000 agg. in 10 hrs.	
13......	1 : 50 agg. in 30 min.	1: 50 agg. in 40 min.	Typhoid fever
	1 : 1,000 agg. in 5 hrs.	1: 1,000 agg. in 8 hrs.	
14......	1 : 50 agg. in 20 min.	1: 30 agg. in 30 min.	Typhoid fever
	1 : 2,000 agg. in 5 hrs.	1: 2,000 agg. in 9 hrs.	
15......	1 : 50 agg. in 10 min.	1: 50 agg. in 15 min.	Typhoid fever
	1 : 4,000 agg. in 6 hrs.	1: 4,000 agg. in 8 hrs.	
16......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 500 agg. in 6 hrs.	1: 500 agg. in 8 hrs.	
17......	1 : 50 no agg.	1: 50 no agg.	Paratyphoid
18......	1 : 50 agg. in 5 min.	1: 50 agg. in 5 min.	Typhoid fever
	1 : 20,000 agg. in 10 hrs.	1: 20,000 agg. in 12 hrs.	
19......	1 : 50 agg. in 40 min.	1: 50 agg. in 1 hr.	Typhoid fever
	1 : 500 agg. in 5 hrs.	1: 500 agg. in 7 hrs.	
20......	1 : 50 agg. in 10 min.	1: 50 agg. in 15 min.	Typhoid fever
	1 : 10,000 agg. in 6 hrs.	1: 10,000 agg. in 8 hrs.	
21......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 500 agg. in 3 hrs.	1: 500 agg. in 6 hrs.	
22......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 800 agg. in 5 hrs.	1: 800 agg. in 8 hrs.	
23......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 1,000 agg. in 6 hrs.	1: 1,000 agg. in 10 hrs.	
24......	1 : 50 no agg.	1: 50 no agg.	Tuberculosis
25......	1 : 50 agg. in 10 min.	1: 50 agg. in 15 min.	Typhoid fever
	1 : 10,000 agg. in 3 hrs.	1: 10,000 agg. in 5 hrs.	
26......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 500 agg. in 6 hrs.	1: 500 agg. in 9 hrs.	
27......	1 : 50 agg. in 20 min.	1: 50 agg. in 30 min.	Typhoid fever
	1 : 200 agg. in 5 hrs.	1: 200 agg. in 8 hrs.	
28......	1 : 50 agg. in 10 min.	1: 50 agg. in 15 min.	Typhoid fever
	1 : 5,000 agg. in 8 hrs.	1: 5,000 agg. in 12 hrs.	
29......	1 : 50 agg. in 1 hr.	1: 50 agg. in 1½ hrs.	Typhoid fever
	1 : 100 agg. in 6 hrs.	1: 100 agg. in 10 hrs.	

TABLE OF RESULTS—*Continued.*

Case	Dead Bacilli	Living Bacilli	Remarks
30.. ...	1:50 agg. in 1 hr. 1:80 agg. in 5 hrs.	1:50 agg. in 1½ hrs. 1:80 agg. in 8 hrs.	Typhoid fever
31......	1:50 agg. in 1 hr. 1:100 agg. in 6 hrs.	1:50 agg. in 1½ hrs. 1:100 agg. in 8 hrs.	Typhoid fever
32......	1:50 agg. in 1 hr. 1:100 agg. in 5 hrs.	1:50 agg. in 2 hrs. 1:100 agg. in 7 hrs.	Typhoid fever
33......	1:50 agg. in 40 min. 1:1,000 agg. in 5 hrs.	4:50 agg. in 1 hr. 1:1,000 agg. in 8 hrs.	Typhoid fever
34......	1:50 agg. in 1 hr. 1:200 agg. in 5 hrs.	1:50 agg. in 2 hrs. 1:200 agg. in 9 hrs.	Typhoid fever

majority of these cases was established also by the isolation of the typhoid bacillus in cultures of the blood; two proved to be paratyphoid by bacteriologic examination of the blood; one doubtful; and the other tuberculosis.

In comparing the results obtained with dead cultures with those obtained with living cultures, it was found that the two differ very slightly. It was, however, noticed that the reaction becomes evident somewhat sooner with the dead than with the living cultures. This is especially noticeable in high dilutions which require some hours for the agglutination to become visible to the naked eye. Dried blood dissolved in distilled water gives practically the same results as fresh serum. Although the dried-blood method is very convenient for clinical purposes, one can save time and trouble by using fresh blood, which gives practically the same results as fresh serum or dried blood. Four drops of pure blood are allowed to flow into a small test-tube or vial containing 2 c.c. of a 1:500 solution of formalin in distilled water. Distilled water lakes the blood, forming a clear solution. The formalin is added to prevent bacterial growth in case some time elapses before the blood is tested. This gives approximately a 1:10 solution of fresh serum.

SUMMARY.

In summing up, briefly, the steps of the method most suitable for daily clinical use are as follows:

1. Inoculate a large quantity of plain bouillon (100–1,000 c.c.) with B. typhosus, incubate at 36° C. for twenty-four hours, and

add 1 c.c. formalin for every 100 c.c. of bouillon. The culture is now ready for future use, except that it must be shaken before it is used, because the dead organisms gradually settle to the bottom. A culture prepared in this manner is always ready for use, and can be kept at room temperature for many months.*

2. With a lancet or some other sharp instrument prick the lobe of the ear or the finger tip (preferably the lobe of the ear) and collect four drops of blood in a small test-tube or vial containing 2 c.c. of a 1:500 solution of formalin in distilled water. Laking is soon complete, making a clear solution of approximately 1:10.

3. To 1 c.c. of the blood solution add 4 c.c. of the dead culture, making a dilution of about 1:50, and set the tube aside.

4. Make a control tube by mixing 1 c.c. of a 1:10 solution of normal blood, or 1 c.c. of distilled water, with 4 c.c. of the culture, and set it aside.

If agglutination takes place, the dead organisms collect in clumps, and within an hour or two are seen as a flocculent precipitate settling toward the bottom, leaving the bouillon clear at the end of twelve to twenty-four hours.

Dried blood may be used instead of fresh blood. On a glass slide collect four drops of blood, spread it, and allow it to dry. When wanted for use, dissolve it in 2 c.c. of distilled water and proceed as directed above.

Among some of the general deductions from this and other recent work on the serum test in typhoid fever, the following seem worthy of special stress: By keeping on hand and using typhoid cultures killed by formalin, one is always prepared to apply the serum-test without the use of a microscope or other apparatus outside of one or two small test-tubes or bottles. The time consumed is practically nothing, and the method appears to be suitable for hospitals, boards of health, and private practice.

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*On August 20, 1903, a good reaction was obtained with a culture killed August 20, 1902 — that is, one year after having been killed.